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Award Number: DAMD17-02-2-0001

TITLE: Effect of Pyridostigmine on the Physiologic and Morphologic Changes Induced by Soman at the Human Neuromuscular Junction

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REPORT DATE: January 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	January 2004	Annual (10 Dec 2002 - 9 Dec 2003)

4. TITLE AND SUBTITLE
Effect of Pyridostigmine on the Physiologic and Morphologic Changes Induced by Soman at the Human Neuromuscular Junction

5. FUNDING NUMBERS
DAMD17-02-2-0001

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

Pre-treatment of American troops with pyridostigmine bromide (PB) has been advocated as an effective way to counteract the potential lethal effect of nerve-agent exposure during military operations. This policy is based on experimental evidence indicating that PB provides partial protection to animals exposed to soman. The protecting mechanism of PB involves a temporary inhibition of the end-plate acetylcholinesterase (AChE) which prevents an ensuing irreversible inhibition induced by a nerve agent. Human studies have been limited to the use of the erythrocyte AChE inhibition as a surrogate marker of PB efficacy. However, it remains unproven that the level of erythrocyte AChE activity is a reliable indicator of the protection provided by PB against nerve agents in humans.

This application proposes to use intracellular microelectrode studies, electron microscopy, and chemical assessment of the AChE to study the effects of soman on the neuromuscular junction (NMJ) of human intercostal muscles pre-exposed to PB. The muscle specimens will be donated by consenting patients undergoing thoracic surgery at the University of California, Davis Medical Center.

This study may provide very important and relevant data that may direct future policies regarding the PB pretreatment of military personnel under the threat of nerve-agent exposure.

14. SUBJECT TERMS

15. NUMBER OF PAGES
10

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Annual Report, December 2003: DAMD17-02-2-0001

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INTRODUCTION

"Effect of Pyridostigmine on the Physiologic and Morpohologic Changes Induced by Soman at the Human Neuromuscular Junction"

The treatment of soman exposure consists of a combination of pyridostigmine bromide (PB) given preventively before the exposure to soman, and atropine-pralidoxime chloride therapy administered following contact with the nerve agent.

The protecting mechanism of PB is thought to depend on the temporary inhibition of the end-plate acetylcholinesterase (AChE) which partially precludes a subsequent irreversible inhibition induced by soman.

While there are sufficient experimental results in animals that support the protective mechanism of PB, there is no comparable data in humans that support the putative protective effect of PB.

Recently, the FDA has approved the use of PB in military personnel based solely on experimental animal results ("animal rule"); however, the FDA has requested the US Army Medical Research and Materiel Command provide experimental results in humans and attach the data as "post-marketing information."

BODY

There are three fundamental problems with the data that are currently available supporting the preventive use of PB in humans. (1) The erythrocyte acetylcholinesterase inhibition, that has been presented as a supportive evidence of the use of PB in humans, has not been clearly established as a surrogate end-point for the clinical benefit of PB. (2) There is lack of data correlating PB benefit and acetylcholinesterase inhibition at a more relevant site, such as the human neuromuscular junction. (3) There is lack of evidence showing that PB benefits humans.

An additional problem is that a pivotal, non-human primate study involving animal exposure to soman failed to show a dose-dependent correlation between survival and pre-exposure to PB.

The specific aim of the proposed in-vitro study is to demonstrate that the degree of acetylcholinesterase inhibition induced by pre-exposure of human muscles to PB correlates with the amelioration of the physiological and structural changes induced by soman. In a subsequent study a link can be established between the degree of acetylcholinesterase inhibition in erythrocytes and at the human neuromuscular junction in subjects receiving PB for at least three days (steady-state).

The proposed study involves the use of human intercostal muscles obtained from consenting patients undergoing thoracic surgery. The muscles will be dissected into a layer of few muscle fibers and will be either exposed to several concentrations of PB ranging from 10^{-6} to 10^{-7} or not exposed to PB. Subsequently, all the muscle preparations (those pre-incubated and those not pre-incubated with PB) will be exposed to soman in a concentration in the range of $4x10^{-7}$ M. A group of muscles will be either exposed only to PB or neither exposed to PB nor to soman and will serve as controls. After washing out both PB and soman the muscles will be divided in three segments; one for physiologic studies (measurement of tension and microelectrode recordings), another one for structural studies involving electron microscopy of the neuromuscular junction and another one for chemical assessment of the end-plate AChE.

KEY RESEARCH ACCOMPLISHMENTS

1. The protocol was approved by the Office of Human Research Protection (OHRP) at UC Davis in February 2003.

2. The USAMRMC decided not to resubmit the protocol for approval by the Human Subject Research Review Board (HSRRB) at the Defense Department until having a new meeting with FDA.

3. Due to the military operations taken place during the second quarter of the year, the meeting with FDA was delayed until June 2003.

4. The meeting with FDA was held on June 16. The FDA agreed with the experimental design stated in the description of study approved by the OHRP at UC Davis on February 2003.

5. A re-submission was sent to the HSRRB, and the protocol was reviewed on September 10. The HSRRB approved the protocol conditionally, pending minimal changes.

6. A second re-submission was sent on October 29.

7. The protocol was approved by the HSRRB on November 7.

8. The protocol was submitted to the OHRP at UC Davis on December 5.

9. The protocol was evaluated by the board on December 17.

10. The protocol was favorably reviewed and approved by the OHROat UC Davis, pending minimal modifications.

REPORTABLE OUTCOMES

None

CONCLUSIONS

The FDA has agreed with the design of the study, and the protocol has been approved by the HSRRB and by the OHRP, pending minimal modifications. The research could be started during 2004 as soon as the OHRP provides the final approval of the protocol.

REFERENCES

None

APPENDICES

None